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(54) Monacolin K derivatives

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None
(58) Field of search
C2C
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(57) Salts and esters of the parent acid corresponding to the lactone Monacolin K may be prepared by salification or esterification of Monacolin K or of a reactive derivative thereof and the salts may be prepared by cultivation of various fungi of the genus *Monascus* under appropriate conditions. These salts and esters have an activity greater than or comparable with that of Monacolin K itself in the inhibition of biosynthesis of cholesterol. They are, accordingly, valuable antihypercholesteraemic agents.

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FIG. 1

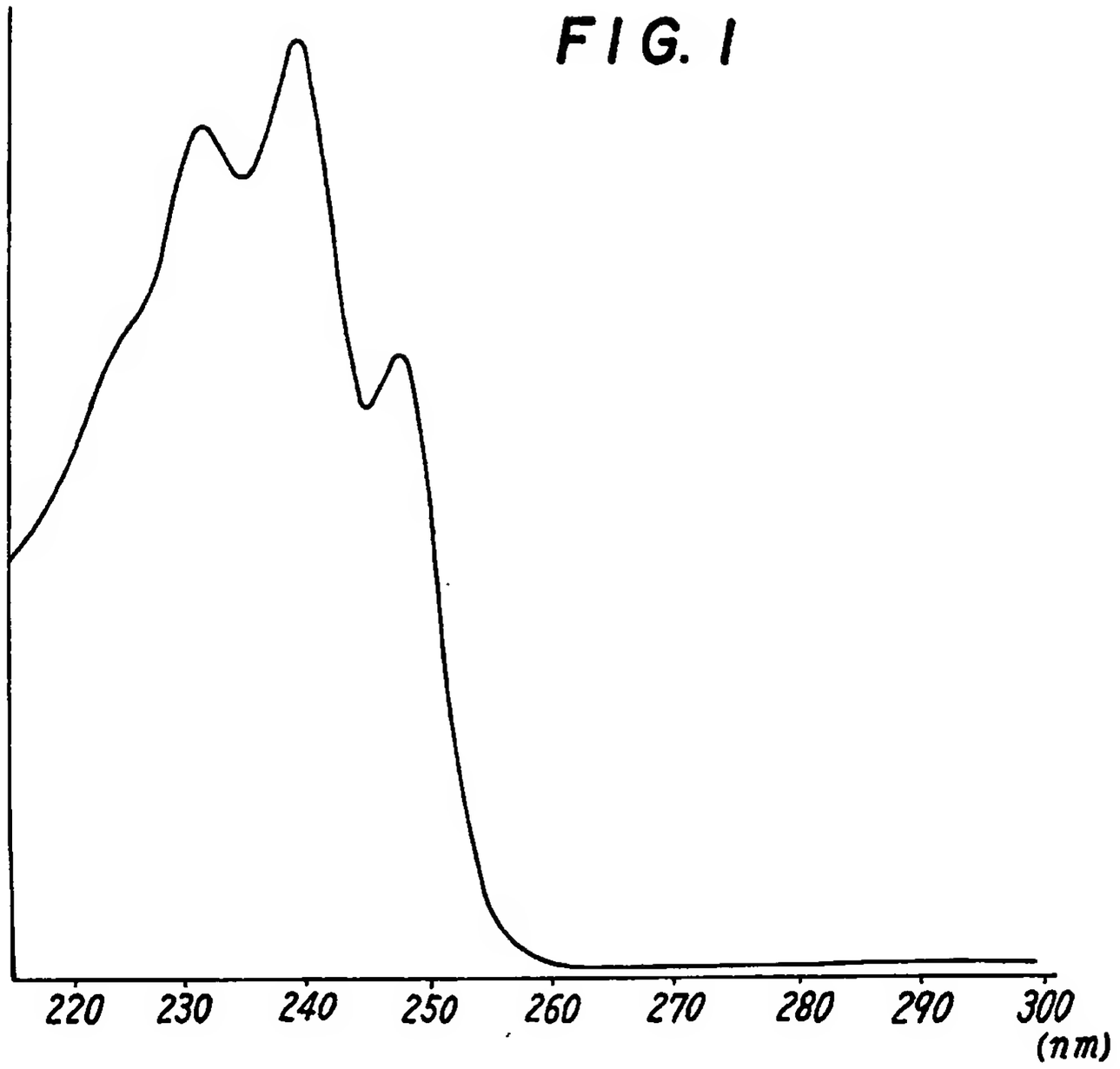


FIG. 2

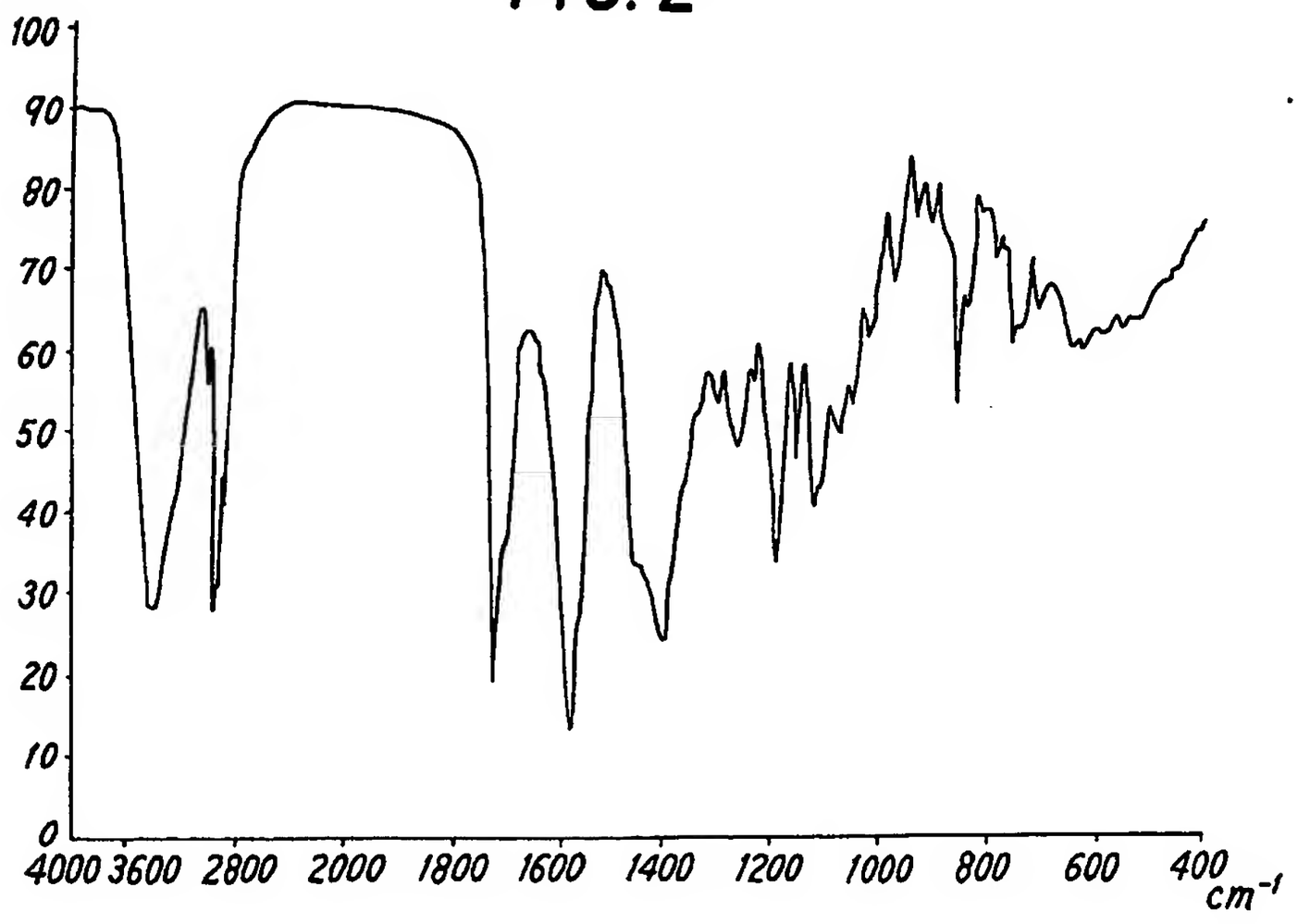
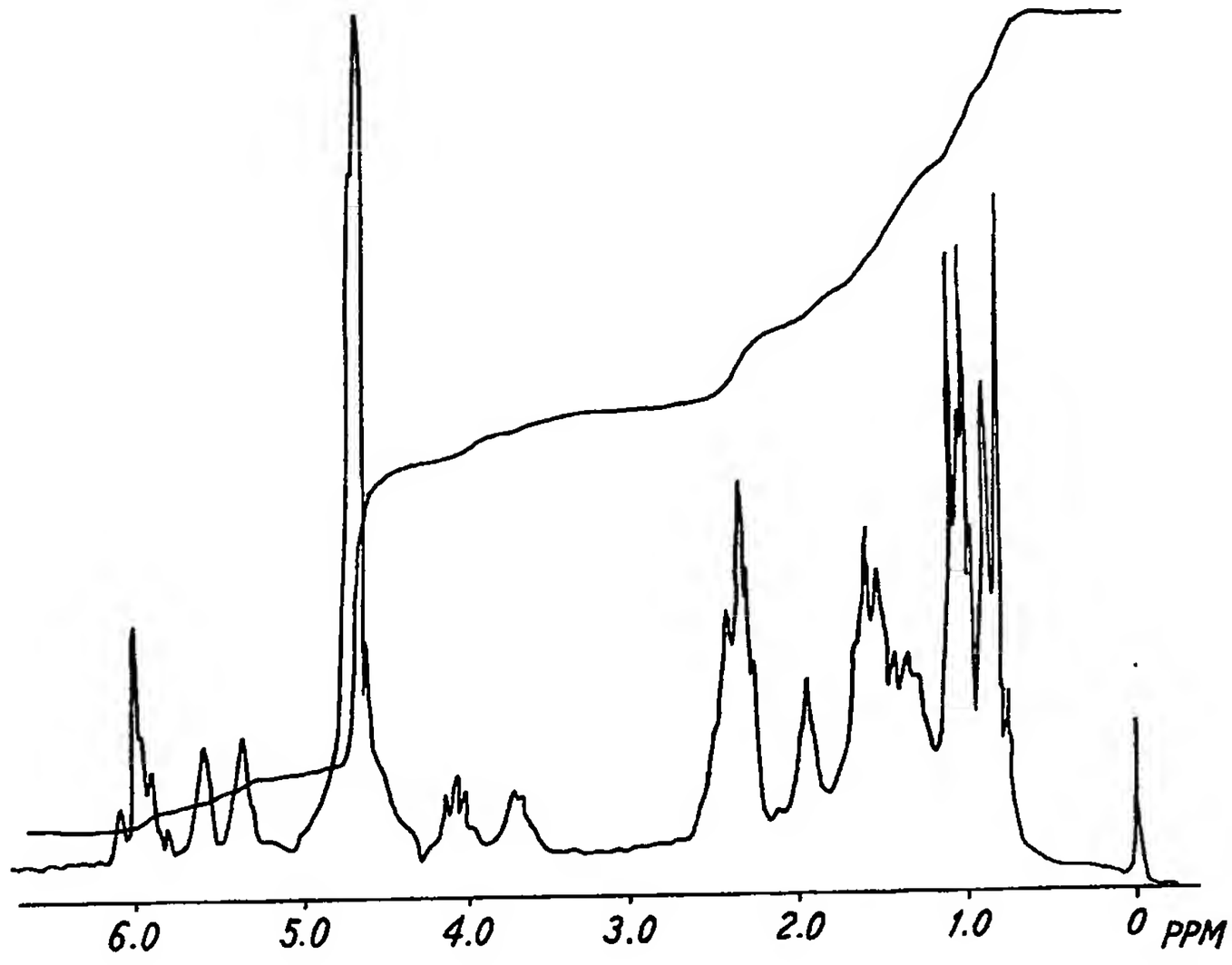


FIG. 3



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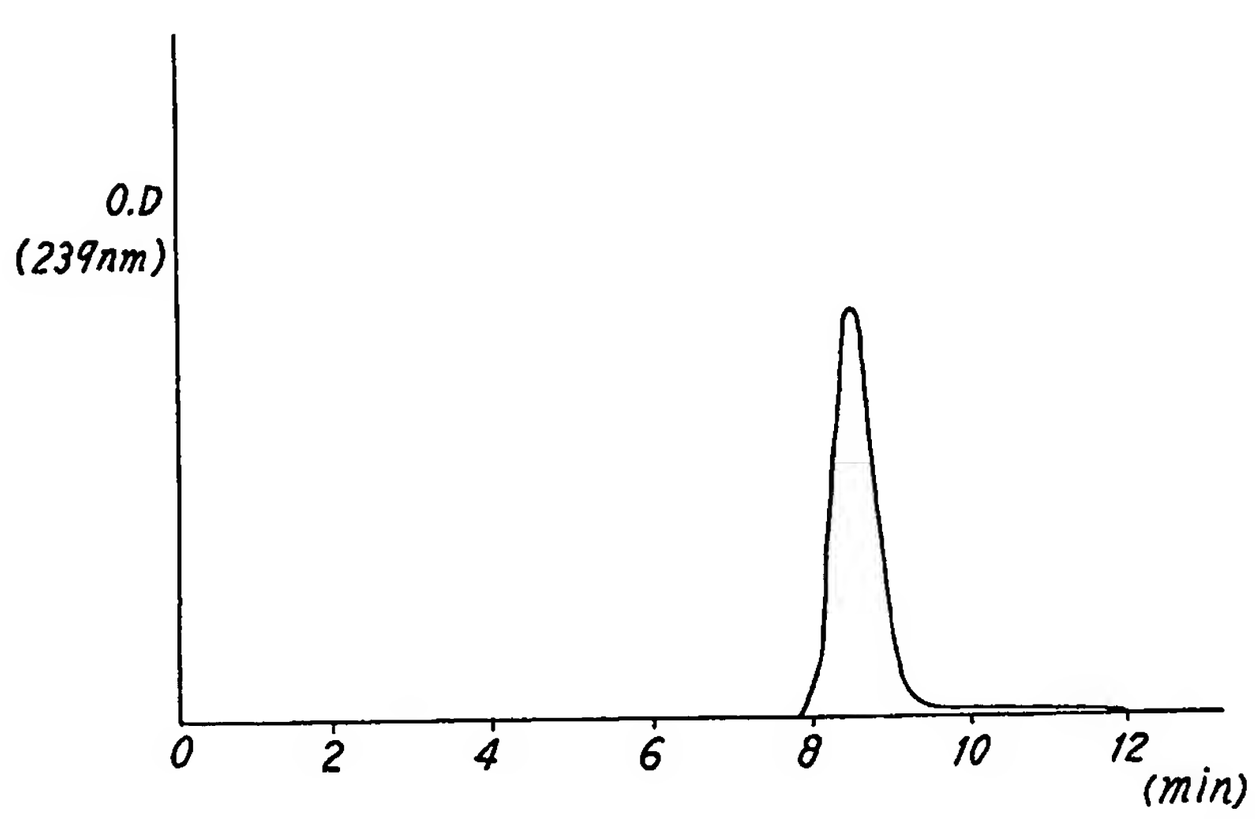

FIG. 5

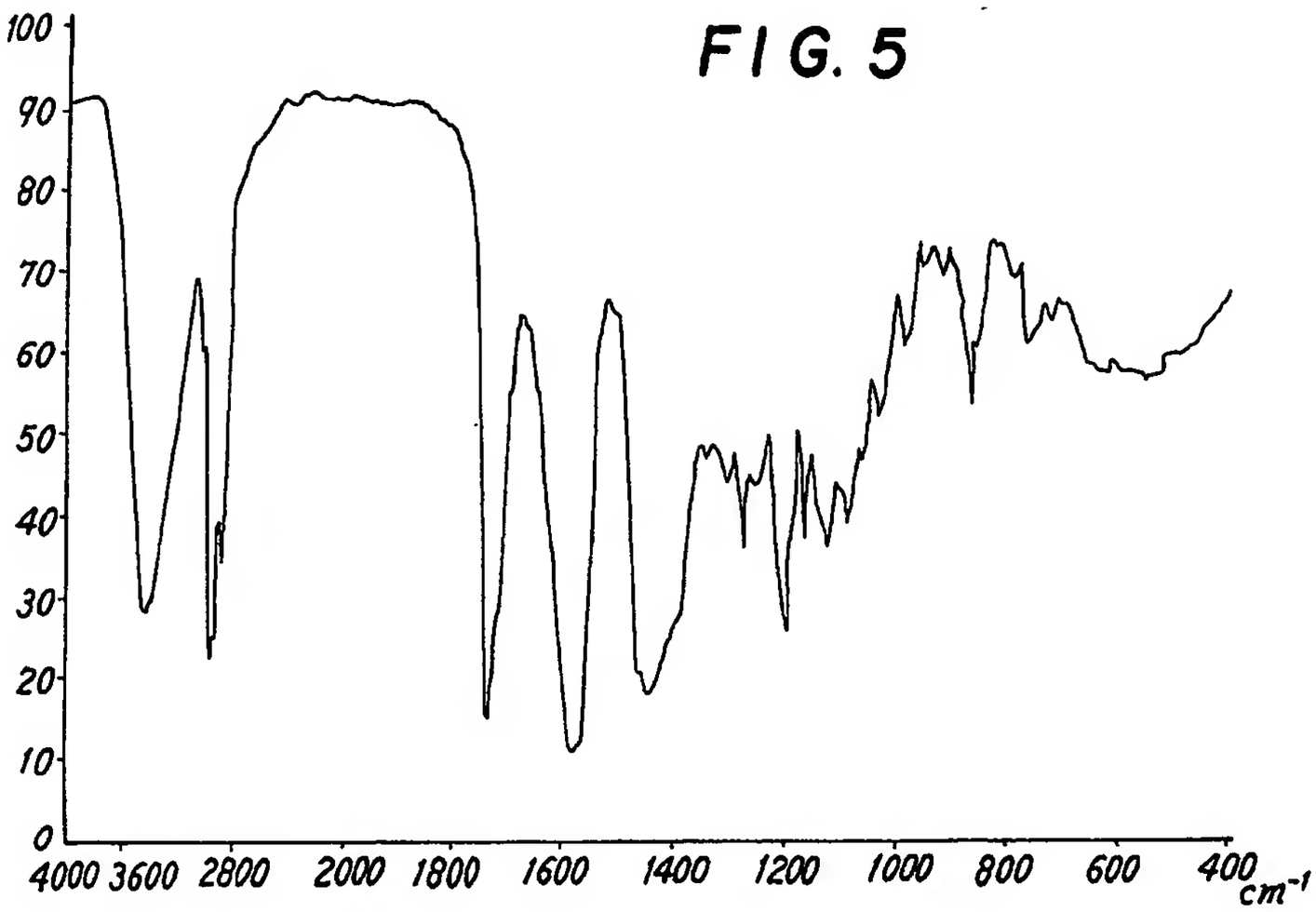
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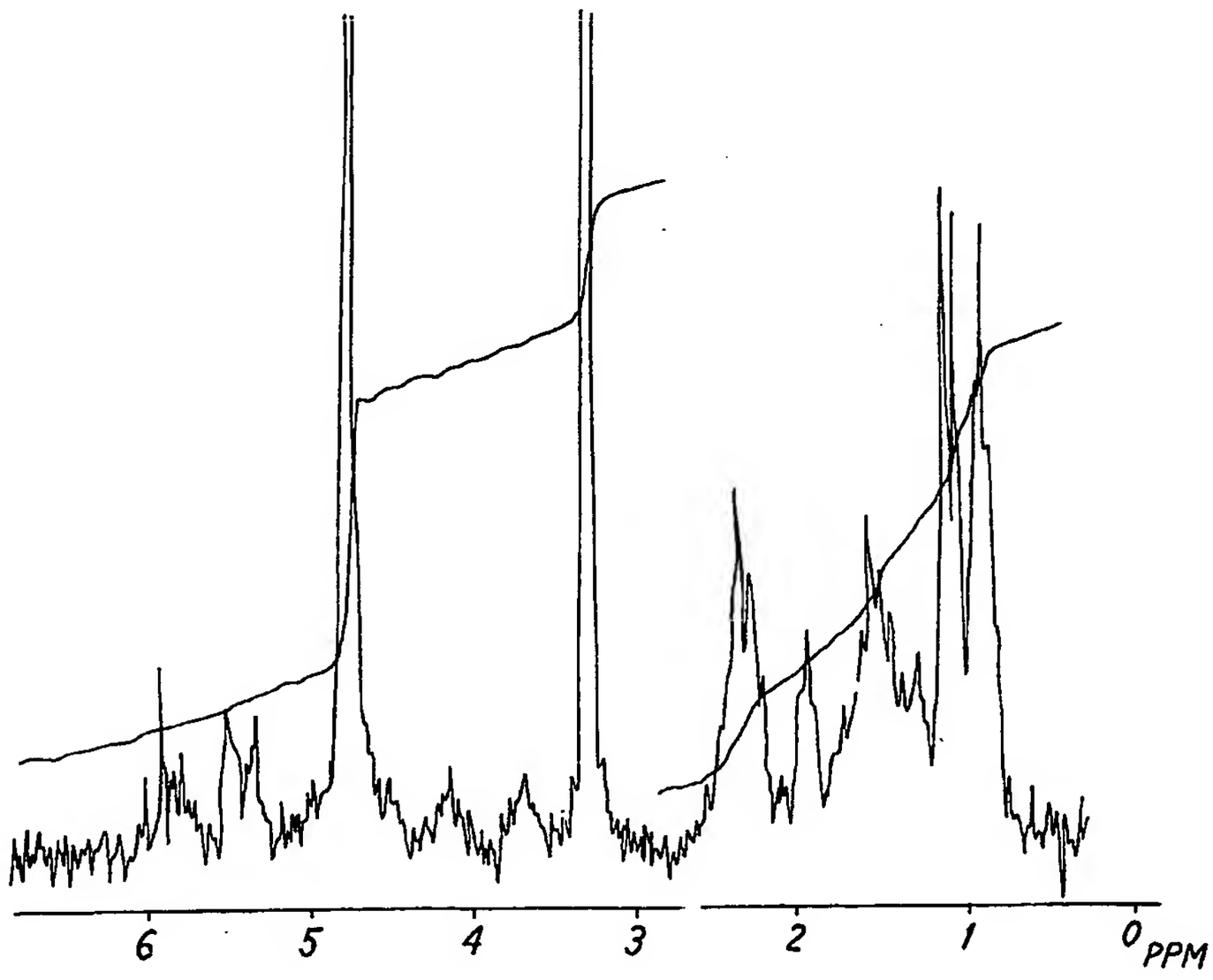
FIG. 6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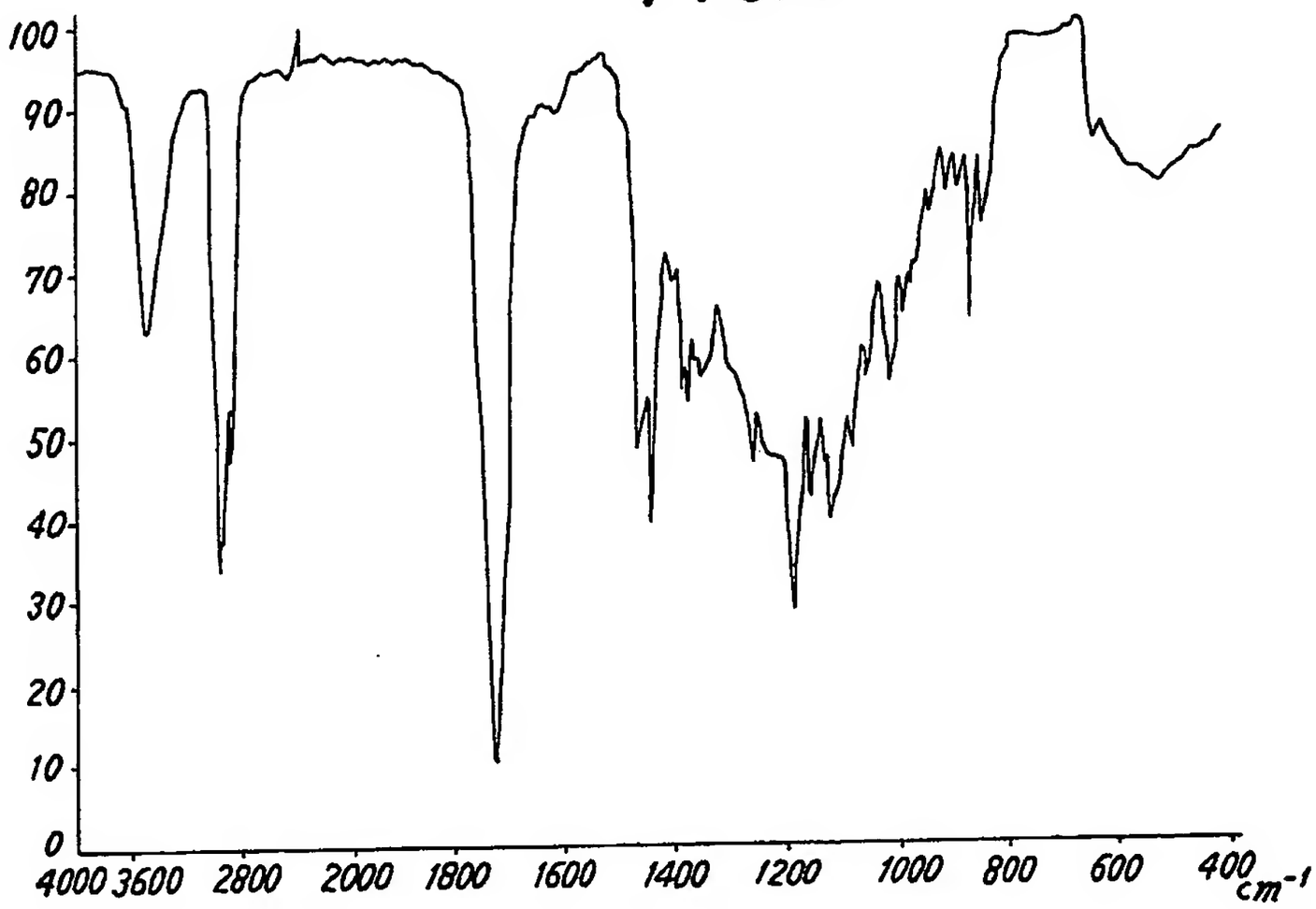
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FIG. 8

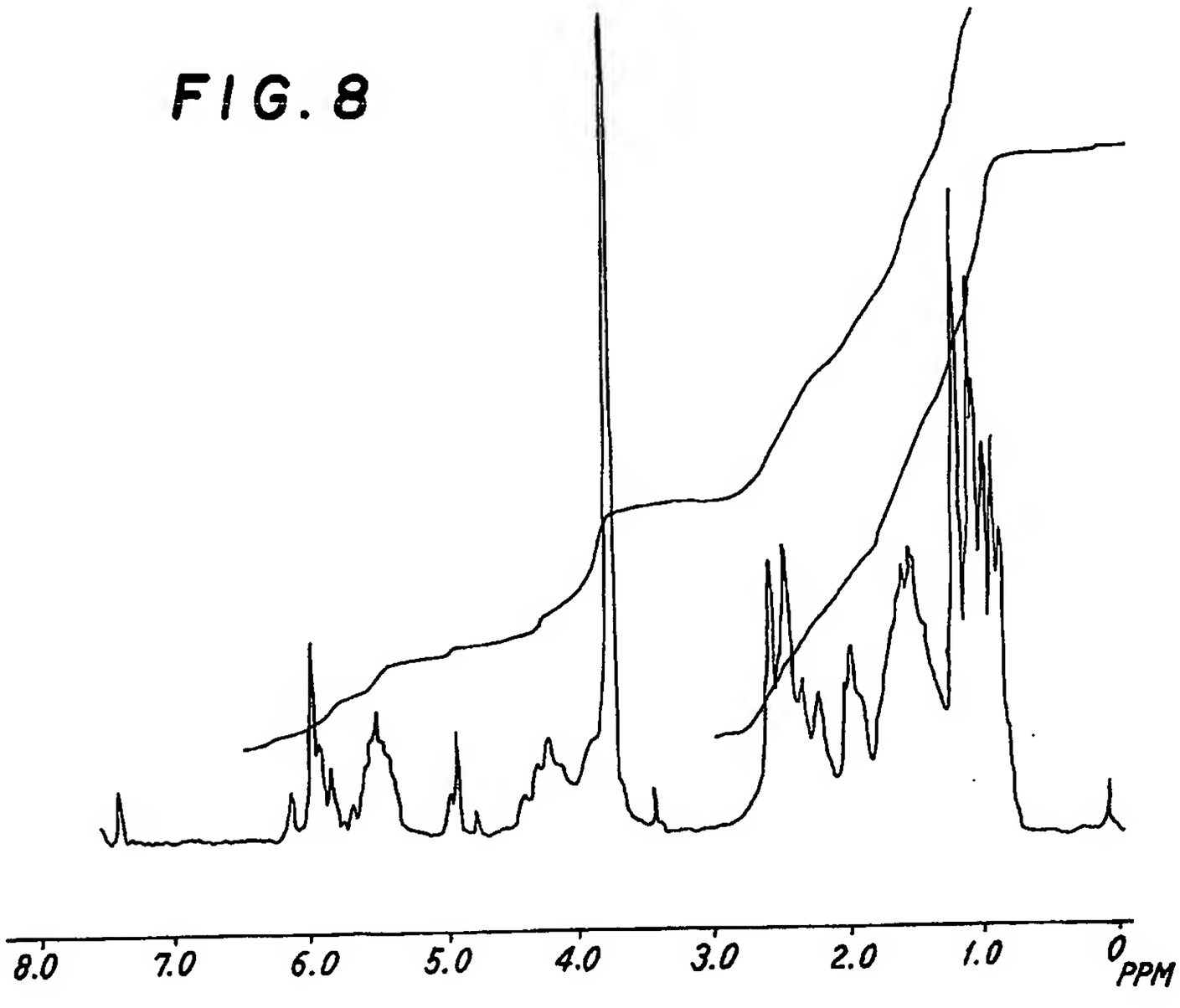
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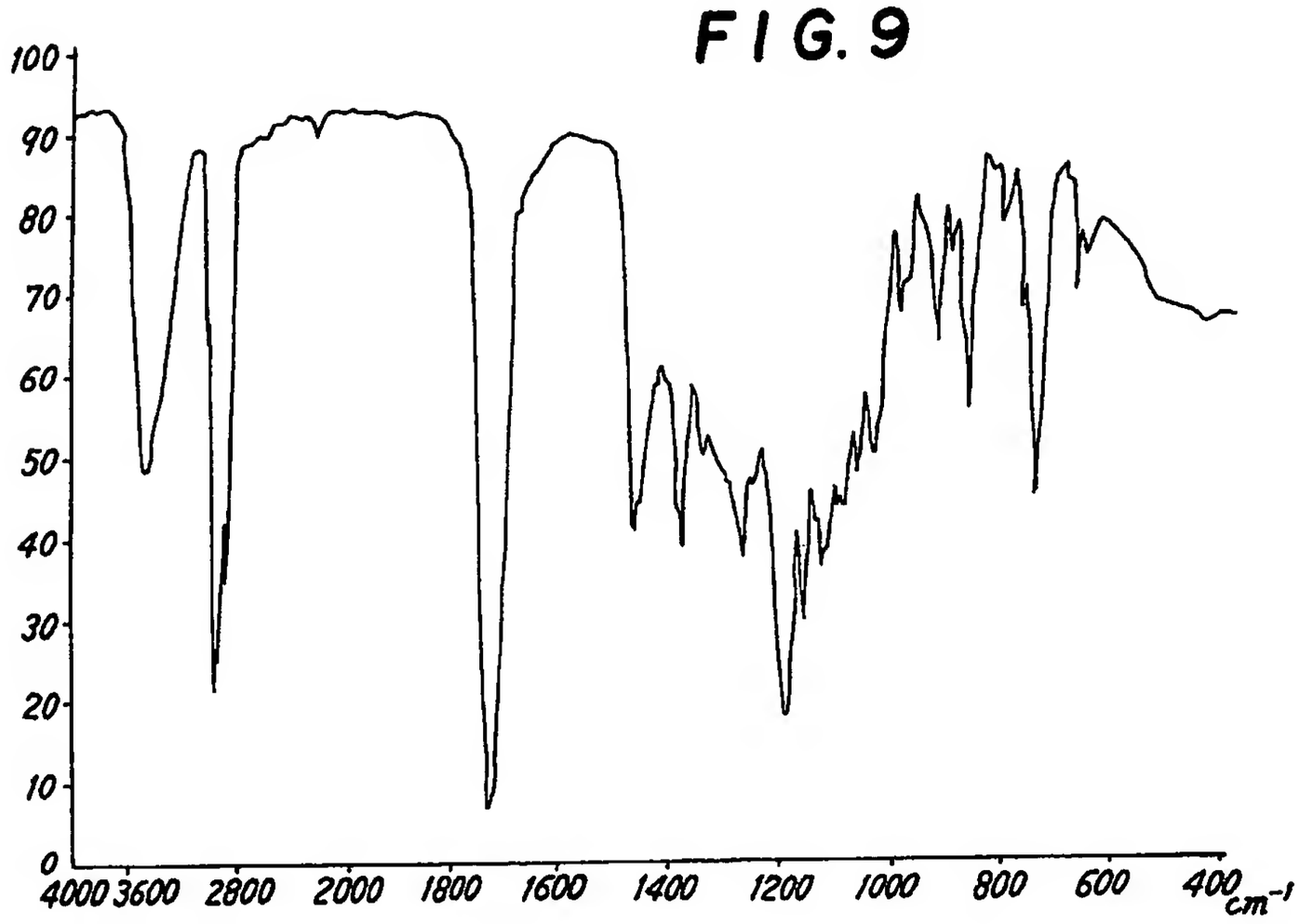
FIG. 9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c.m⁻¹

FIG. 10

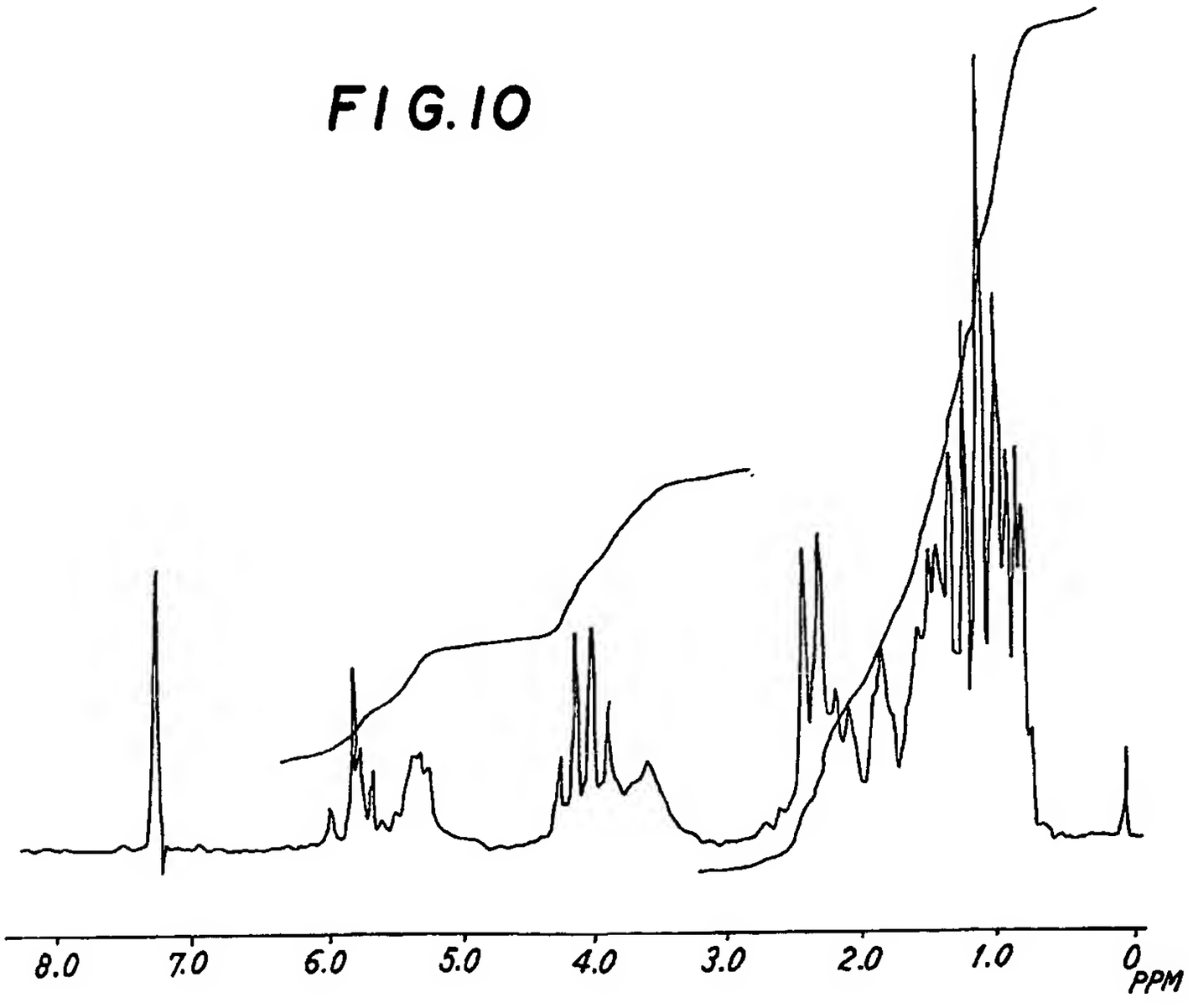
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FIG. 11

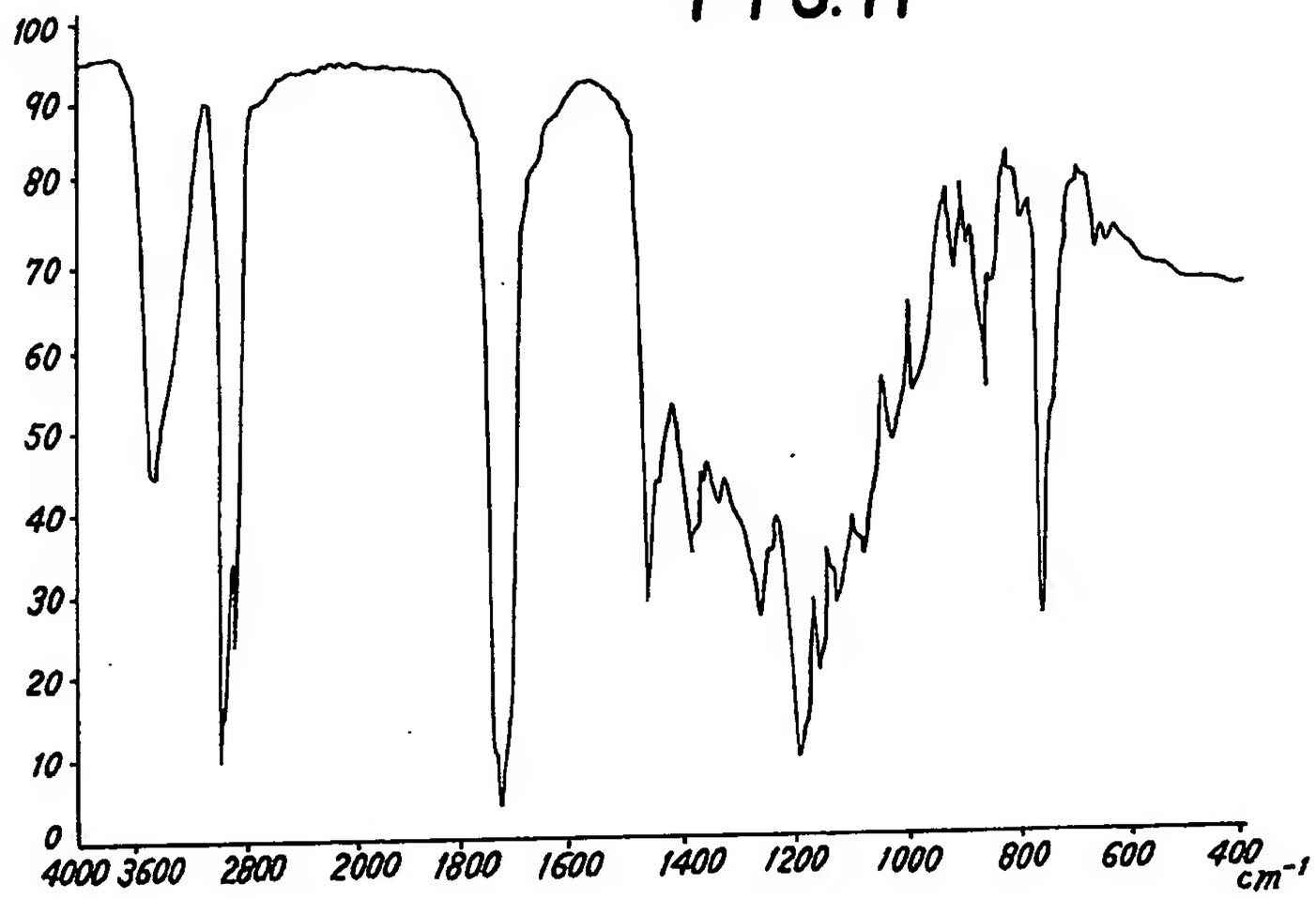

FIG. 12

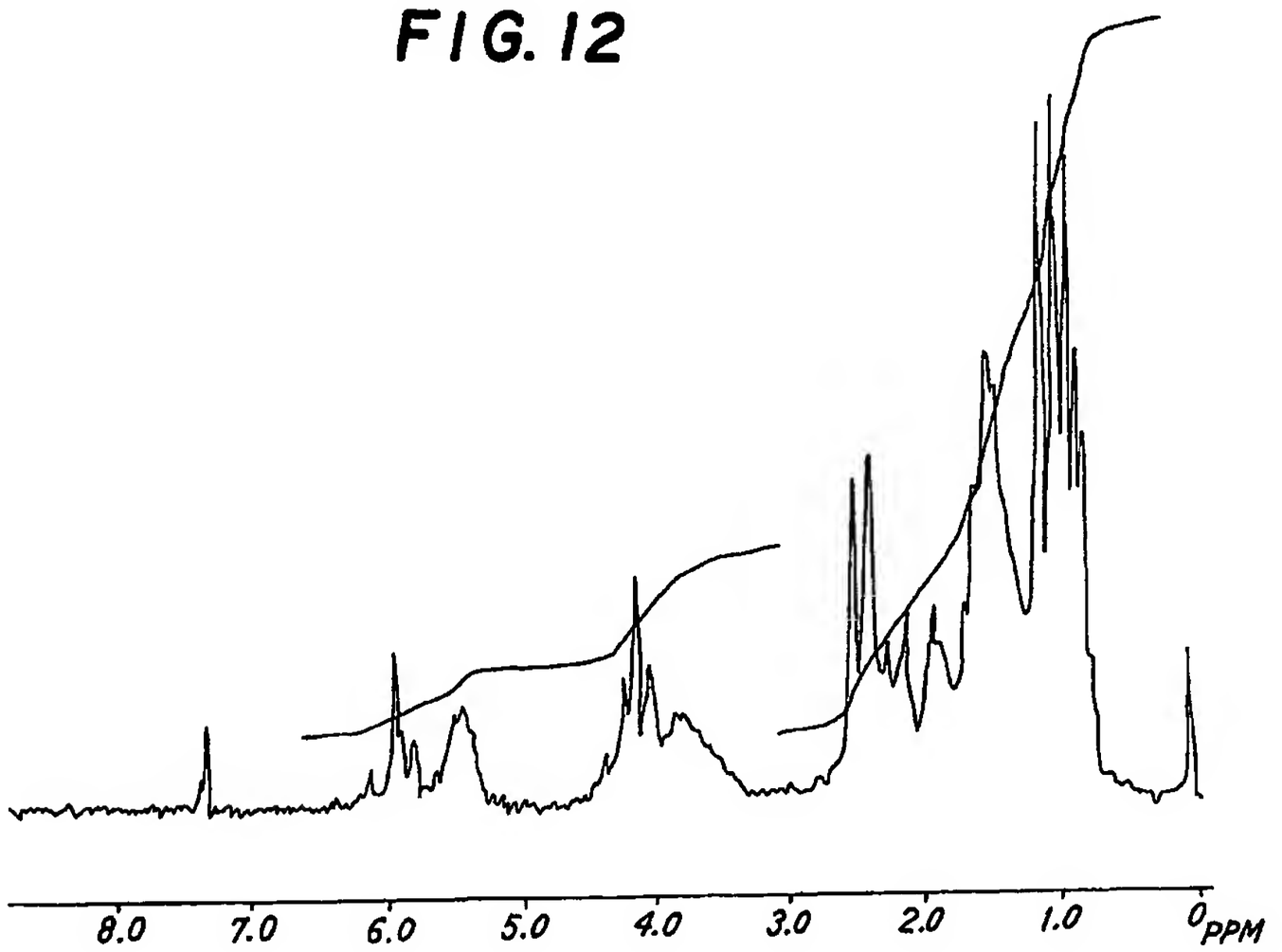

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FIG. 13

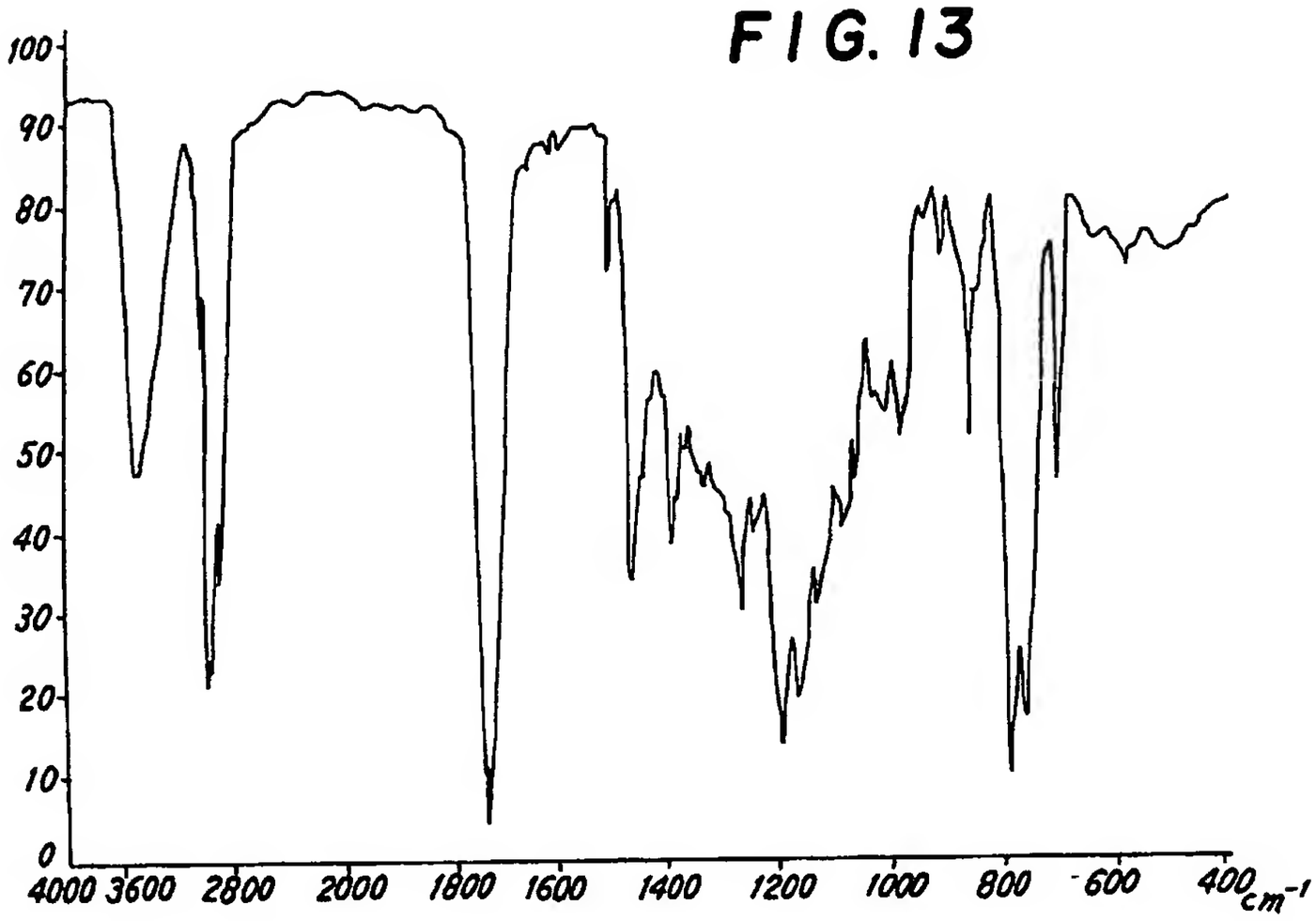
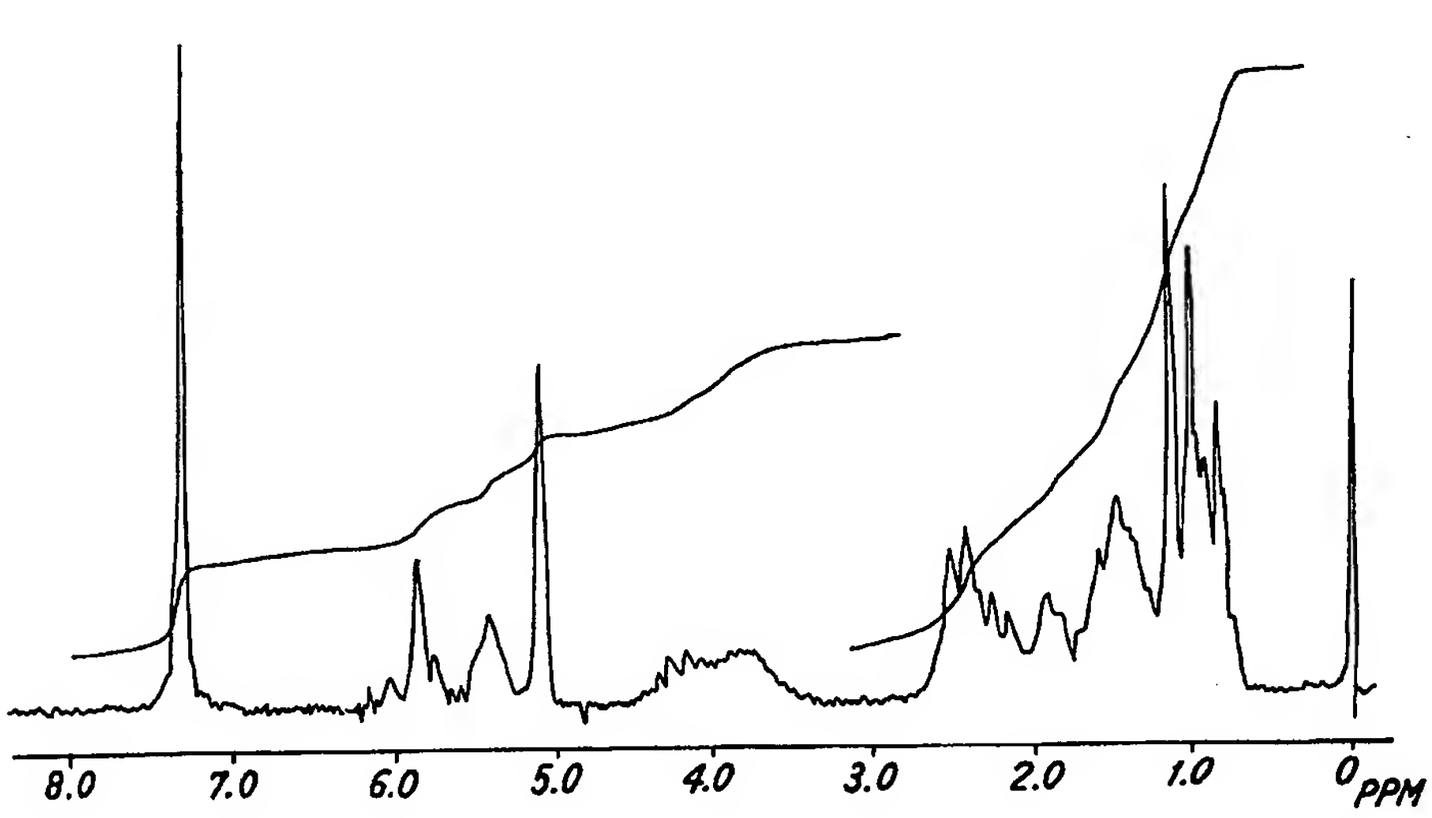


FIG. 14



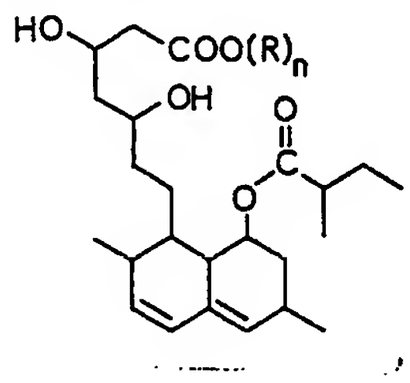
SPECIFICATION

Monacolin K derivatives

The present invention relates to salts and esters of the free acid corresponding to the lactone Monacolin K, to processes for their preparation and to their use as anti-hypercholesteraemic agents.

Our co-pending British Patent Application No. 8005748, discloses the compound Monacolin K and its preparation by microorganisms of the genus *Monascus,* especially *Monascus ruber* strain 1005 (FERM 4822). In this Application, the valuable and unexpected activity of the compound Monacolin K as an anti-hypercholesteraemic agent is disclosed. Subsequently, our co-pending British Patent Application No. 8007240 disclosed the preparation of Monacolin K by the cultivation of a variety of other microorganisms of the genus *Monascus*. We have now found that the salts and esters of the free acid of which Monacolin K is the lactone have an anti-hypercholesteraemic activity of the same type as Monacolin K but to a comparable or greater degree. For convenience, these compounds are hereinafter referred to as "Monacolin K salts or esters" and it will be understood that this expression refers to the salts and esters of the acid of which Monacolin K is the lactone.

The Monacolin K salts and esters provided by the present invention have the formula:



in which:

R represents a substituted or unsubstituted alkyl group or a metal atom; and

n is the reciprocal of the valency of the group or atom represented by R.

The invention also provides a process for preparing Monacolin K salts and esters by the salification or esterification of Monacolin K or a reactive derivative thereof.

The invention still further provides a process for preparing salts of Monacolin K by cultivating a Monacolin K salt-producing microorganism of the genus *Monascus* and separating a Monacolin K salt from the culture medium.

The Monacolin K salts to which the present invention applies are metal salts and preferably: alkali metal salts, such as sodium or potassium salts; alkaline earth metal salts, such as the calcium or magnesium salts; salts of metals from Group IIIa of the Periodic Table of the Elements, such as the aluminium salt, and salts of transition metals from Groups Ib, IIb and VIII of the Periodic Table of the Elements, such as the iron, nickel, cobalt, copper and zinc salts. Of these metal salts, the alkali metal salts, alkaline earth metal salts and aluminium salt are preferred and the sodium, calcium and aluminium salts are most preferred.

Monacolin K salts can readily be converted to Monacolin K itself or to the parent acid of Monacolin K by acidification. The resulting Monacolin K or acid will revert to a salt in the presence of an alkali e.g. an alkali metal hydroxide or carbonate. This exchange can be carried out quantitatively and repeatedly. We have found that conversion between Monacolin K or its parent acid and the metal salt is closely related to the pH of the medium containing them. The critical pH value is about 5.0. Provided that metal ions are available, the Monacolin K is always present in the form of a salt when the pH is above 5.0. On the other hand, where the pH is below 5.0. Monacolin K itself, the parent acid of Monacolin K or a mixture of the two in varying ratios will be present.

The Monacolin K esters provided by the present invention are those compounds having the above formula in which R represents a substituted or unsubstituted alkyl group. Where R represents an unsubstituted alkyl group, it may be a straight or branched chain group preferably having up to 8 carbon atoms. Examples of such alkyl groups represented by R include the methyl, ethyl, propyl, isopropyl, butyl and hexyl groups.

Where R represents a substituted alkyl group, the substituent may, as is well-known in the art, be selected from a wide range of groups and atoms, including aryl groups, acyl groups (especially arylcarbonyl groups), alkoxy groups, halogen atoms and hydroxy groups. Particularly preferred substituents are aryl groups and arylcarbonyl groups, that is to say R represents an aralkyl group or an arylcarbonylalkyl group.

Where R represents an aralkyl group, it is preferably a substituted or unsubstituted benzyl group. Substituted benzyl groups preferably have one or more substituents selected from alkyl groups, alkoxy groups and halogen atoms. Examples of such benzyl groups include the benzyl, 2-methylbenzyl, 3-methylbenzyl, 4-methylbenzyl, 2-ethylbenzyl, 3-ethylbenzyl, 4-ethylbenzyl, 2-methoxybenzyl, 3-methoxybenzyl, 4-methoxybenzyl, 2-ethoxybenzyl, 3-ethoxybenzyl, 4-ethoxybenzyl, 2-chlorobenzyl, 3-chlorobenzyl, 4-chlorobenzyl, 2-bromobenzyl, 3-bromobenzyl or 4-bromobenzyl groups.

Where R represents an arylcarbonylalkyl group, it is preferably a substituted or unsubstituted phenacyl group. Substituted phenacyl groups preferably have one or more substituents selected from alkyl groups, alkoxy groups and halogen atoms. Examples of such phenacyl groups include the phenacyl, 2-methylphenacyl, 3-methylphenacyl, 4-methylphenacyl, 2-ethylphenacyl, 3-ethylphenacyl, 4-ethylphenacyl, 2-methoxyphenacyl, 3-methoxyphenacyl, 4-methoxyphenacyl, 2-ethoxyphenacyl, 3-ethoxyphenacyl, 4-ethoxyphenacyl, 2-chlorophenacyl, 3-chlorophenacyl, 4-chlorophenacyl, 2-bromophenacyl, 3-bromophenacyl or 4-bromophenacyl groups.

Of the esters, we particularly prefer the methyl, ethyl, butyl and benzyl esters.

The Monacolin K salts and esters of the present

invention may readily be prepared by salification or esterification of Monacolin K itself or of a reactive derivative of Monacolin K. Examples of suitable reactive derivatives include the parent acid of Monacolin K and, in the case of the preparation of esters, Monacolin K salts (e.g. those classes of salt described above, especially the sodium, potassium, calcium, magnesium, aluminium, iron, zinc, copper, nickel or cobalt salt).

Monacolin K salts may be prepared by simple reaction of Monacolin K or its parent acid with an oxide, hydroxide, carbonate or bicarbonate, preferably a hydroxide or carbonate, of the chosen metal. Care should be taken to ensure that the reaction is carried out at a pH value exceeding 5.0 and preferably exceeding 7.0 to ensure complete conversion of the Monacolin K or acid to the desired salt. The Monacolin K employed in this reaction will preferably be prepared, as described in our aforementioned co-pending Applications, by cultivation of a fungus of the genus *Monascus* and isolation of Monacolin K from the culture medium. The Monacolin K may be subjected to isolation from the culture medium and purification prior to salification or, more preferably, the salification reaction is effected in the course of the isolation and purification of Monacolin K from the culture medium, so that the Monacolin K is isolated in the form of its desired metal salt. Whichever method is adopted, the metal salt may be isolated from the reaction medium or the culture medium by methods well-known in the art, including those hereafter described in connection with the isolation of Monacolin K metal salts from cultures of Monacolin K salt-producing fungi.

Monacolin K esters may be prepared by simple esterification of Monacolin K or a reactive derivative thereof. The reaction may be carried out by reacting an alcohol of formula ROH (or a reactive derivative thereof) in which R represents a substituted or unsubstituted alkyl group with Monacolin K or its parent acid, preferably in the presence of a dehydrating agent, e.g. an acid halide, such as acetyl chloride. Alternatively, the Monacolin K esters may be prepared by reacting a Monacolin K salt with a halide of formula RX (in which R represents a substituted or unsubstituted alkyl group and X represents a halogen atom, preferably an iodine atom).

It is also possible to prepare Monacolin K salts directly by the cultivation of a Monacolin K salt-producing microorganism of the genus *Monascus*. Suitable microorganisms which can be used in this process include: *Monascus anka* SANK 10171 (IFO 6540); *Monascus purpurous* SANK 10271 (IFO 4513); *Monascus ruber* SANK 10671 (Ferm 4958); *Monascus vitreus* SANK 10960 (NIHS 609, e-609; Ferm 4960); *Monascus paxii* SANK 11172 (IFO 8201); *Monascus ruber* SANK 11272 (IFO 9203); *Monascus ruber* SANK 13778 (Ferm 4959); *Monascus ruber* SANK 15177 (Ferm 4956); *Monascus ruber* SANK 17075 (CBS 832.70); *Monascus ruber* SANK 17175 (CBS 503.70); *Monascus ruber* SANK 17275 (ATCC 18199); and *Monascus ruber* SANK 18174 (Ferm 4957). All of these microorganisms are available from recognized culture collections, as indicated by the following codes:

IFO = Institute for Fermentation, Osaka, Japan;

FERM = Fermentation Research Institute, Agency of Industrial Science and Technology, Ministry of International Trade and Industry, Japan;

NIHS = National Institute of Hygenic Sciences, Japan;

CBS = Centraal Bureau voor Schimmel-cultures, Netherlands;

ATCC = American Type Culture Collection, Maryland, U.S.A..

Preferred strains of the genus *Monascus* which may be used in the process of the invention to produce Monacolin K salts are *Monascus ruber* SANK 10671, *Monascus ruber* SANK 11272, *Monascus ruber* SANK 13778, *Monascus ruber* SANK 15177 and *Monascus ruber* SANK 18174. *Monascus ruber* SANK 10671, *Monascus ruber* SANK 13778, *Monascus ruber* SANK 15177 and *Monascus ruber* SANK 18174 are all fungi recently isolated from soil by the present inventors and their microbiological properties are given below:

Monascus ruber SANK 15177 (FERM 4956)

This strain was isolated from soil at Tukimino, Yamato-city, Kanagawa-prefecture, Japan and was deposited on 27 April 1979 under the accession No. 4956 with the said Fermentation Research Institute.

The strain grows well on a potato-glucose-agar medium at 25°C and produces a soluble colouring material having a yellowish-brown to reddish-brown colour in the medium. It forms many cleistothecia on the basal layer of hyphae.

On oatmeal agar medium, it produces a pale brown colouring material and grows well. Formation of cleistothecia is good and the cleistothecia are spherical, of diameter 30-60 microns and formed on short stalks. These stalks are nearly colourless and branched and of size 25-60 x 3.5-5.0 microns. The asci are evanescent and thus difficult to observe. The ascospores are colourless and ellipsoid and their dimensions are 4.5-6.5 x 4.0-5.0 microns, their surfaces are smooth. The conidia are linked basipetally and are of size 7.0-10.0 x 6.0-10.0 microns. Their tissues are disrupted.

Although the strain will grow at 37°C, best growth is observed between 23 and 30°C.

Monascus Ruber SANK 10671 (FERM 4958)

This strain was isolated from soil at Shinagawa-ku, Tokyo, Japan and was deposited on 27 April, 1979 with the said Fermentation Research Institute under the accession No. 4958.

Growth on potato-glucose-agar and oatmeal agar media is similar to that of strain SANK 15177, except that the soluble colouring matter produced is dark red. The diameter of the cleistothecia is 30-80 microns and the dimensions of the stalks are 30-70 x 3.0-5.0 microns. Asci are not observed. The ascospores are colourless and ellipsoid and their dimensions are 4.5-6.5 x 4.0-5.0 microns. The conidia are colourless and pyriform or ovoid and their dimensions are 6.0-10.0 x 6.0-8.5 microns.

Monascus ruber SANK 13778 (FERM 4959)

This strain was isolated from soil at Inawashiro-cho, Nagat, Yama-gun, Fukushima-prefecture, Japan and was deposited on 27 April 1979 under the accession No. 4959 with the said Fermentation Research

Institute.

Growth on potato-glucose-agar and oatmeal agar media is similar to that of strain SANK 15177, except that the soluble colouring material produced is a pale reddish-brown to reddish-brown colour. The cleistothecia are of diameter 35-75 microns and the stalks are 30-70 x 3.5-5.0 microns. Asci are not observed. The ascospores are colourless and ellipsoid and their dimensions are 4.5-6.0 x 4.0-5.0 microns; their surfaces are smooth. The dimensions of the conidia are 7.0-10.0 x 6.0-10.0 microns.

Monascus ruber SANK 18174 (FERM 4957)

This strain was isolated from soil at Shakotan-cho, Shakotan-gun, Shiribeshi Shicho, Hokkaido-prefecture, Japan and was deposited on 27 April, 1979 with the said Fermentation Research Institute under the accession No. 4957.

Growth on potato-glucose-agar and oatmeal agar media is similar to that of strain SANK 15177, except that the colouring material produced is pale pink. The cleistothecia are of diameter 20-70 microns and the dimensions of the stalks are 20-60 x 3.0-5.0 microns. Asci are not observed. The ascospores are colourless and ellipsoid and their dimensions are 5.0-7.0 x 4.0-5.5 microns; their surfaces are smooth. The conidia are linked together basipetally and are colourless and most of them are pyriform and of dimensions 6.0-9.5 x 6.0-10.0 microns.

Based on the observations of their characteristics as reported above, these microorganisms were all identified as strains of *Monascus ruber* van Tieghem.

Microbiological properties of *Monascus ruber* have been reported in the following literature: Takada, Transaction of the Micological Society of Japn, *9*, 125-130 (1969) [Materials for the Fungus Flora of Japan (7)] and van Tieghem, Bull. Soc. Botan. France, *31* 227 (1884). Ascospore generation of the strain has been reported by Cole *et al* in the Canadian Journal of Botany, *46*, 987 (1968), "Conidium ontogeny in hyphomycetes. The imperfect state of *Monascus ruber* and its meristem arthrospores".

Apart from the strains of fungus mentioned above, any fungi of the genus *Monascus*, including varieties and mutants, which are capable of producing Monacolin K salts may be employed in the process of the present invention.

Monacolin K salts may be produced by cultivating the chosen microorganism in a culture broth under aerobic conditions, using the same techniques as are well-known in the art for the cultivation of fungi and other microorganisms. For example, the chosen strain of *Monascus* may first be cultivated on a suitable medium and then the produced microorganisms may be collected and innoculated into and cultivated on another culture medium to produce the desired Monacolin K salt; the culture medium used for the multiplication of the microorganism and the culture medium used for the production of the Monacolin K salt may be the same or different. Any culture medium well-known in the art for the cultivation of fungi may be employed, provided that it contains, as is well-known, the necessary nutrient materials, especially as assimilable carbon source and an assimilable nitrogen source. Examples of suitable sources of assimilable carbon are glucose, maltose, dextrin, starch, lactose, sucrose and glycerine. Of these sources, glucose and glycerine are particularly preferred for the production of Monacolin K salts. Examples of suitable sources of assimilable nitrogen are peptone, meat extract, yeast, yeast extract, soybean meal, peanut meal, corn steep liquor, rice bran and inorganic nitrogen sources. Of these nitrogen sources, peptone is particularly preferred. When producing the Monacolin K salt, an inorganic salt and/or a metal salt may, if necessary, be added to the culture medium. Furthermore, if necessary, a minor amount of a heavy metal may also be added. In the production of Monacolin K salts by fermentation with a fungus of the genus *Monascus*, it is important that there should be present in the culture medium or within the body of the fungus metal ions corresponding to the metal salt which it is desired to produce.

We particularly prefer that the microorganism should first be sub-cultured on a potato-dextrose-agar medium (e.g. a product of Difco Company) and then innoculated into and cultivated on another culture medium to produce the desired Monacolin K salt.

The microorganism is preferably cultivated under aerobic conditions using cultivation methods well-known in the art, for example solid culture, shaken culture or culture under aeration and agitation. The microorganism will grow over a wide temperature range, e.g. from 7 to 35°C, but, especially when the microorganism is grown for the purpose of producing Monacolin K or a Monacolin K salt, the more preferred cultivation temperature is within the range of 20 to 30°C.

During the cultivation of the microorganism, the production of the Monacolin K salt may be monitored by sampling the culture medium and measuring the physiological activity of the Monacolin K salt in the culture medium by the tests described hereafter. Cultivation may then be continued until a substantial accumulation of Monacolin K salt has been achieved in the culture medium, at which time the Monacolin K salt may then be isolated and recovered from the culture medium and the tissues of the microorganism by any suitable combination of isolation techniques, chosen having regard to its physical and chemical properties. For example, any or all of the following isolation techniques may be employed: extraction of the liquor from the culture broth with a hydrophilic solvent (such as diethyl ether, ethyl acetate or chloroform); extraction of the organism with a hydrophilic solvent (such as acetone or an alcohol); concentration, e.g. by evaporating off all or part of the solvent under reduced pressure; dissolution into a more polar solvent (such as acetone or an alcohol); removal of impurities with a less polar solvent (such as petroleum ether or hexane); gel filtration through a column of a material such as Sephadex (a trade name for a material available from Pharmacea Co. Limited, U.S.A.); absorptive chromatography with active carbon or silica gel; rapid liquid chromatography; conversion to Monacolin K itself or its parent acid; direct purifica-

tion in the form of the metal salt; and other similar methods. By using a suitable combination of these techniques, the desired Monacolin K salt can be isolated from the culture broth as a pure substance.

As described in our aforementioned co-pending Applications, Monacolin K itself may also be produced using the microorganisms and techniques described above.

The physiological activity of Monacolin K salts and esters can be assayed and determined quantitatively by the following tests, which can also be employed, in modified form, to monitor the production of Monacolin K salts in the course of the fermentation process of the present invention.

1. Inhibition of cholesterol biosynthesis

Like Monacolin K itself, Monacolin K salts and esters specifically inhibit the activity of 3 - hydroxy - 3 - methylglutaryl - CoA reductase, which is the rate-determining enzyme in the biosynthesis of cholesterol. The following Table 1 gives the concentrations (in ng/ml) of the compounds of the invention inhibiting the activity of 3 - hydroxy - 3 - methylglutaryl (HMG)-CoA reductase by 50% [as measured by the method described in Analytical Biochemistry, *31*, 383 (1969)] and the concentrations (in ng/ml) of the compound of the invention inhibiting cholesterol biosynthesis by 50% [as measured by the method of the Journal of Biological Chemistry, *247*, 4914 (1972)]. Also given are the corresponding results for the known compounds ML-236B (which is a compound known to have a similar type of activity and produced by cultivating microorganisms of the genus *Penicillium*, as disclosed in our United Kingdom Patent Specification No. 1,453,425). Also given is the concentration of Monacolin K itself which inhibits cholesterol biosynthesis by 50%. It will be seen that, whereas the concentration of ML-236B required to inhibit cholesterol biosynthesis by 50% is 10.0 ng/ml, the corresponding concentration for the Monacolin K esters is of the order of 1 ng/ml (i.e. about 10 times better) and the corresponding concentration for the sodium salt of Monacolin K is about 0.14 ng/ml (i.e. about 70 times better). The activities of the salts and esters of Monacolin K are comparable with or better than that of Monacolin K itself.

Table 1

Compound	*Concentration (ng/ml) required to inhibit by 50%*	
	HMG-CoA reductase	*cholesterol biosynthesis*
methyl ester	70.0	1.2
ethyl ester	12.0	1.3
butyl ester	15.0	2.0
benzyl ester	11.0	1.1
sodium salt	2.0	0.14
calcium salt	12.0	1.8
Monacolin K	—	2.0
ML - 236B	10.0	10.0

2. Reduction in blood and liver cholesterol levels

The animals used in this test were rats of the Wistar Imamichi strain, each having a body weight of about 300 g. The tests were conducted on groups of rats, each group consisting of 5 animals. Each animal was intravenously injected with 400 mg/mg of Triton WR-1339 (a trade name for a material known to increase the blood cholesterol level) whilst simultaneously administering orally one of the compounds shown in following Table 2 in the amount shown in that Table. 20 hours after oral administration, the rats were sacrificed by bleeding and the blood and livers were collected and their cholesterol levels were determined by conventional means. The results are reported in Table 2, which gives the reduction in blood and liver cholesterol levels compared with a control group of rats to which Triton WR-1339 alone had been administered.

For purposes of comparison, the corresponding results are given for Monacolin K itself and for ML-236B, but these compounds were administered in doses substantially greater than those used for the compounds of the invention in order to achieve a comparable reduction in cholesterol levels.

Table 2

Compound	*Dose mg/kg*	*Reduction in cholesterol levels (%) in the*	
		blood	*liver*
methyl ester	5	23.8	18.4
ethyl ester	5	23.3	18.0
butyl ester	5	19.5	15.7
benzyl ester	5	24.4	18.5
sodium salt	2	27.5	18.9
calcium salt	5	21.4	17.1
Monacolin K	10	22.4	16.7
ML-236-B	40	24.6	20.9

The reduction in blood or liver cholesterol level was given by the formula:

$$\frac{1-(C-B)}{A-B} \times 100$$

where:

A = level in group treated only with Triton WR-1339;
B = level in untreated control group;
C = level in test group.

3. Acute toxicity

The compounds tested were the methyl, ethyl, butyl and benzyl esters and sodium and calcium salts of Monacolin K. It was found that the 50% lethal dose (LD_{50}) of each of these compounds was at least 2000 mg/kg on oral administration and at least 500 mg/kg on intraperitoneal administration. The compounds thus have a very low toxicity.

These results demonstrate that the compounds of the invention inhibit the biosynthesis of cholesterol and, as a result, lower cholesterol levels in blood.

They are thus valuable medicines in the treatment of hyperlipaemia and arteriosclerosis.

The compounds of the invention may be administered orally or parenterally in the form of a capsule, tablet, injectable preparation or any other known formulation, although we normally prefer to administer them orally. The dose will vary, depending upon the age and body weight of the patient and the severity of the condition, but, in general, the daily dose for an adult is preferably from 0.1 to 100 mg, more preferably from 0.1 to 10 mg, in the case of Monacolin K salts and from 0.5 to 100 mg, more preferably from 0.5 to 10 mg, in the case on Monacolin K esters.

The preparation of the compounds of the invention is further illustrated by reference to the following Examples.

EXAMPLE 1

Sodium salt of Monacolin K

300 litres of a culture medium having a pH of 5.5 before sterilization and containing 5% w/v glucose, 0.5% w/v corn steep liquor, 2% w/v peptone (Kyokuto brand, available from Kyokuto Seiyaku KK, Japan) and 0.5% w/v ammonium chloride was charged into a 600 litre fermenter and innoculated with the organism *Monascus ruber* SANK 18174 (Ferm. 4957). Cultivation of the organism was effected for 116 hours at 26°C with an aeration rate of 300 litres per minute and agitation at 190 revolutions per minute. At the end of this time, the pH of the culture broth containing the organism was adjusted to a value of 3.4 by the addition of 6N hydrochloric acid and then the broth was extracted with 800 liters of methanol.

6 kg of Hyflo Super Cel were added and then the extract was filtered, using a filter press, to give 1100 litres of methanolic extract. This extract was washed with 200 litres of a saturated aqueous solution of sodium chloride and then with 180 litres of ethylcyclohexane. The resulting solution was extracted with 600 litres of ethylene dichloride. 50 g of trifluoroacetic acid were added to the ethylene dichloride extract and allowed to react at 80°C for 30 minutes. The reaction mixture was then washed successively with 200 litres of a 2% w/v aqueous solution of sodium bicarbonate and with 200 litres of a 10% w/v aqueous solution of sodium chloride. The mixture was then concentrated by evaporation under reduced pressure to give 135 g of an oily substance.

This oily substance was dissolved in 400 ml of methanol. 20 ml of the resulting methanolic solution (containing 6.8 g of the oil) were then subjected to preparative rapid liquid chromatography using a Waters Co. Limited System 500 equipped with a Prepac C_{18} column (reversed phase column). A 85 : 15 by volume mixture of methanol and water was used as the developer. Development was carried out at a flow rate of 200 ml/minute (developing time about 10 minutes), watching a differential refractometer connected to the body and the fraction showing the main peak on the differential refractometer was separated. This operation was repeated and the resulting main peak fractions were collected and concentrated to give 10.2 g of an oily substance. This oily substance was dissolved in 30 ml of methanol, and 6 ml of the methanolic solution (containing about 2 g of the oil) were subjected again to the same preparative rapid liquid chromatography and developed with a 80 : 20 by volume mixture of methanol and water at a flow rate of 20 ml/minute. The fraction showing the main peak was separated. This operation was repeated and the main peak fractions were collected and concentrated. The residue was treated with aqueous methanol to give 1170 mg of crude crystals, which were recrystallized several times from ethanol to give 864 mg of crystals.

To these crystals were added 19.4 ml of 0.1N aqueous sodium hydroxide and the mixture was stirred at 50-60°C for 3 hours. Insolubles were filtered off and the filtrate was lyophilized to give 900 mg of the sodium salt of Monacolin K having the following properties:

1. Colour and form: White powder.
2. Elemental analysis:
   Calculated: C, 64.84%; H, 8.38%, Na, 5.17%.
   Found: C, 64.78%; H, 8.55%; Na, 5.21%.
3. Molecular weight:
   444 [by mass spectrometry (F.D. Mass.)].
4. Molecular formula:
   $C_{24}H_{37}O_6Na$.
5. Ultraviolet absorption spectrum:
   As shown in Figure 1 of the accompanying drawings, λ_{max}:
   232 nm ($\log \epsilon = 4.30$);
   239 nm ($\log \epsilon = 4.36$);
   248 nm ($\log \epsilon = 4.19$).
6. Infrared absorption spectrum (KBr pellet:
   As shown in Figure 2 of the accompanying drawings.
7. Nuclear magnetic resonance spectrum (D_2O):
   As shown in Figure 3 of the accompanying drawings.
8. Rapid liquid chromatography:
   Holding time: 8.5 minutes;
   Column, Microbondapac C18;
   60% v/v aqueous methanol + 0.1% PIC-A (a product of Waters Co. Limited);
   Flow rate, 1.5 ml/minute.
   As shown in Figure 4 of the accompanying drawings.
9. Solubility:
   Soluble in water; insoluble in organic solvents.
10. Specific rotation:
    $[\alpha]_D^{25} = +266°$ (c = 0.33, water).

EXAMPLE 2

Sodium salt of Monacolin K

300 litres of a culture medium having a pH of 7.4 before sterilization and containing 1.5% w/v soluble starch, 1.5% w/v glycerine, 2.0% w/v fish meal and 0.2% w/v calcium carbonate were charged into a 600 litre fermenter and innoculated with the organism *Monascus ruber* SANK 17075 (CBS 832.70). Cultivation of the organism was effected for 120 hours at 26°C with an aeration rate of 300 litres per minute and agitation at 190 revolutions per minute. The culture broth was then filtered using a filter press, to give 35 kg (wet weight) of the organism.

To this were added 100 litres of water and the pH value of the mixture was adjusted to 12 by addition of sodium hydroxide with stirring. The mixture was then left at room temperature for 1 hour, after which

2 kg of Hyflo Super Cel were added to the mixture, which was then filtered using a filter press. The pH of the filtrate was adjusted to a value of 10 by the addition of hydrochloric acid, and the resulting mixture was then adsorbed on a column containing 5 litres of HP-20 resin washed with 15 litres of each of water and a 10% v/v aqueous solution of methanol. The column was then eluted with 90% v/v aqueous methanol. The eluate was concentrated to a volume of about 10 litres and then its pH was adjusted to a value of 2 by addition of hydrochloric acid and the mixture was extracted with ethyl acetate. The extract was washed with a saturated aqueous solution of sodium chloride, dried over anhydrous sodium sulphate and then concentrated by evaporation to dryness to give 50 g of an oily substance containing Monacolin K acid. To this oily substance was added sufficient methanol to give a total volume of 100 ml. 20 ml of the resulting solution were subjected to preparative rapid liquid chromatography using a reversed phase column (as described in Example 1), eluted with a 20% v/v aqueous solution of methanol (containing 2% acetic acid) at a flow rate of 200 ml/minute. The fraction showing the main peak on the differential refractometer was separated (7-10 minutes). The remaining 80 ml of the methanolic solution was then treated by the same procedure. The resulting main peak fractions were collected, concentrated and extracted with ethyl acetate. The extract was concentrated to dryness after adding heptane, to give 1.2 g of an oily substance.

This oily substance was dissolved in 20 ml of methanol and then subjected to rapid liquid chromatography as described above to give 150 mg of Monacolin K acid. To this were added 2 ml of methanol and 100 ml of water and the resulting solution was adjusted to a pH of 8.0 by addition of 1N aqueous sodium hydroxide to give a clear aqueous solution. This solution was passed through a column containing 10 ml of HP-20 resin and then the column was washed with 100 ml of water and eluted with 80% v/v aqueous methanol. The eluate was lyophilized to give 130 mg of the sodium salt of Monacolin K in the form of a white powder. The properties of the product were identical with those reported for the product of Example 1.

EXAMPLE 3

Calcium salt of Monacolin K

The cultivation, extraction, concentration, preparative rapid liquid chromatography and recrystallization from ethanol described in Example 1 were repeated. 500 mg of the resulting crystals were then dissolved in 50 ml of methylene chloride and the resulting solution was filtered through a millipore filter. 20 ml of a saturated aqueous solution of calcium hydroxide were added to the filtrate and then the mixture was vigorously stirred at room temperature. Whenever the pH of the solution decreased to a value below 8, a further 10 ml portion of a saturated aqueous solution of calcium hydroxide was added and stirring was continued. When the pH no longer decreased (after a total of 50 ml of the aqueous solution of calcium hydroxide had been added), distilled water was added and the whole solution was placed into a separating funnel. The organic phase was collected and the solvent was distilled off. The residue was worked up with heptane and then a powder was obtained by subjecting the mixture to ultrasonic waves. The powder was filtered and dried to give 450 mg of the calcium salt of Monacolin K in the form of a white powder.

This calcium salt had the following properties:

1. Molecular weight:
   882 (by mass spectrometry).
2. Molecular formula:
   $(C_{24}H_{37}O_6)_2.Ca$
3. Melting point:
   155-165°C (with decomposition).
4. Specific rotation:
   $[\alpha]_D^{25} = +209°$ (c = 1.48, chloroform).
5. Infrared absorption spectrum (KBr):
   As shown in Figure 5 of the accompanying drawings.
6. Nuclear magnetic resonance spectrum (CD_3OD):
   As shown in Figure 6 of the accompanying drawings.

EXAMPLE 4

Methyl ester of Monacolin K

The cultivation, extraction, concentration, preparative rapid liquid chromatography and recrystallization from ethanol procedures described in Example 1 were repeated to give 864 mg of Monacolin K. 200 mg of this Monacolin K were dissolved in 20 ml of methanol (which had previously been dehydrated with Molecular Sieve 3A). After adding a few drops of acetyl chloride the solution was stirred at room temperature for 3 hours. The solvent was distilled off and the residue was extracted with 50 ml of ethyl acetate. The extract was washed in turn with a 2% w/v aqueous solution of sodium bicarbonate and a saturated aqueous solution of sodium chloride and then dried over anhydrous sodium sulphate, after which the solvent was distilled off. The residue was adsorbed on a column containing 10 g of silica gel (Wakogel C-100) which had previously been treated with benzene. The fractions eluted with a 6 : 94 by volume mixture of ethyl acetate and benzene were collected and the solvent was distilled off to give 65 mg of the methyl ester of Monacolin K as a colourless oil.

This product had the following properties:

1. Molecular weight:
   436.6 (by mass spectrometry).
2. Molecular formula:
   $C_{25}H_{40}O_6$.
3. Specific rotation:
   $[\alpha]_D^{25} = +208°$ (c = 1.05, methanol).
4. Infrared absorption spectrum:
   As shown in Figure 7 of the accompanying drawings.
5. Nuclear magnetic resonance spectrum:
   As shown in Figure 8 of the accompanying drawings.

EXAMPLE 5

Methyl ester of Monacolin K

The sodium salt of Monacolin K was prepared as described in Example 2. 100 mg of this sodium salt were then dissolved in 2 ml of dimethyl sulphoxide and then 50 μl of methyl iodide were added to the solution. The solution was then refluxed, with stir-

ring, at 40-50°C for 5 hours in a reactor equipped with a reflux condenser. The reaction mixture was then diluted with 5 ml of water and extracted with 10 ml of methylene chloride. The solvent was distilled from the extract and the residue was adsorbed on a column containing 10 g of silica gel (Wakogel C-100) which had previously been treated with benzene. The fractions eluted with a 4 : 96 by volume mixture of ethyl acetate and benzene were collected and the solvent was distilled off to give 35 mg of the methyl ester of Monacolin K in the form of an oil.

EXAMPLE 6

Ethyl ester of Monacolin K

100 mg of Monacolin K (prepared as described in Example 1) were dissolved in 15 ml of ethanol which had previously been dehydrated with Molecular Sieve 3A and a few drops of acetyl chloride were added to the solution. The mixture was then stirred at room temperature for 3 hours, after which the solvent was distilled off and the residue was extracted with 50 ml of ethyl acetate. The extract was washed, in turn, with a 2% w/v aqueous solution of sodium bicarbonate and a saturated aqueous solution of sodium chloride and then dried over anhydrous sodium sulphate. After distilling off the solvent, the residue was adsorbed on a column containing 10 g of silica gel (Wakogel C-100) which had previously been treated with benzene. The fractions eluted with a 6 : 94 by volume mixture of ethyl acetate and benzene were collected and the solvent was distilled off to give 30 mg of the ethyl ester of Monacolin K in the form of a colourless oil.

The properties of this compound were as follows:

1. Molecular weight:
   450.6 (by mass spectrometry).
2. Molecular formula:
   $C_{26}H_{42}O_6$.
3. Infrared absorption spectrum ($CHCl_3$):
   As shown in Figure 9 of the accompanying drawings.
4. Nuclear magnetic resonance spectrum ($CDCl_3$):
   As shown in Figure 10 of the accompanying drawings.

EXAMPLE 7

Butyl ester of Monacolin K

200 mg of Monacolin K (Prepared as described in Example 1) were dissolved in 20 ml of butanol, which had previously been dehydrated with Molecular Sieve 3A. After adding a few drops of acetyl chloride to the solution, the resulting mixture was stirred at room temperature for 2 hours. The solvent was then distilled off and the residue was extracted with 50 ml of ethyl acetate. The extract was washed, in turn, with a 2% w/v aqueous solution of sodium bicarbonate and a saturated aqueous solution of sodium chloride. The solution was then dried over anhydrous sodium sulphate and the solvent was distilled off. The residue was adsorbed on a column containing 10 g of silica gel (Wakogel C-100) which had previously been treated with benzene. The fractions eluted with a 4 : 96 by volume mixture of ethyl acetate and benzene were collected and the solvent was distilled off to give 80 mg of the butyl ester of Monacolin K in the form of a colourless oil.

This compound had the following properties:

1. Molecular weight:
   478.7 (by mass spectrometry).
2. Molecular formula:
   $C_{28}H_{46}O_6$.
3. Infrared absorption spectrum ($CHCl_3$):
   As shown in Figure 11 of the accompanying drawings.
4. Nuclear magnetic resonance spectrum ($CDCl_3$):
   As shown in Figure 12 of the accompanying drawings.

EXAMPLE 8

Benzyl ester of Monacolin K

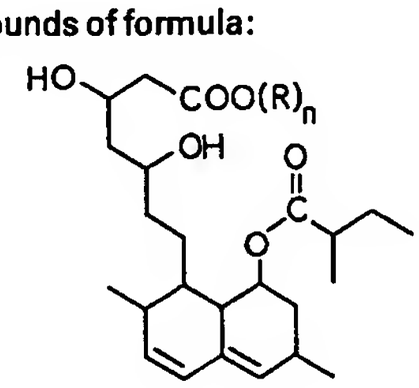
200 mg of Monacolin K (prepared as described in Example 1) were dissolved in 20 ml of benzyl alcohol which had previously been dehydrated with Molecular Sieve 3A. After adding a few drops of acetyl chloride to the solution, the resulting mixture was stirred at room temperature for 3 hours. The solvent was then distilled off and the residue was extracted with 50 ml of ethyl acetate. The extract was washed with, in turn, a 2% w/v aqueous solution of sodium bicarbonate and a saturated aqueous solution of sodium chloride. The solution was then dried over anhydrous sodium sulphate and the solvent was distilled off. The residue was adsorbed on a column containing 10 g of silica gel (Wakogel C-100) which had previously been treated with benzene. The fractions eluted with a 4 : 96 by volume mixture of ethyl acetate and benzene were collected and the solvent was distilled off to give 70 mg of the benzyl ester of Monacolin K in the form of a colourless oil.

This compound had the following properties:

1. Molecular weight:
   512.6 (by mass spectrometry).
2. Molecular formula:
   $C_{31}H_{44}O_6$.
3. Infrared absorption spectrum ($CHCl_3$):
   As shown in Figure 13 of the accompanying drawings.
4. Nuclear magnetic resonance spectrum ($CDCl_3$):
   As shown in Figure 14 of the accompanying drawings.

CLAIMS

1. Compounds of formula:



in which:

R represents a metal atom or a substituted or unsubstituted alkyl group; and

n is the reciprocal of the valency of the atom or group represented by R.

2. Monacolin K salts according to Claim 1, in which R represents a metal atom.

3. Salts according to Claim 2, in which said metal atom is sodium, potassium, calcium, magnesium, aluminium, iron, zinc, copper, nickel or cobalt.

4. Salts according to Claim 2, in which said metal is sodium, calcium or aluminium.

5. Monacolin K esters according to Claim 1, in which R represents a substituted or unsubstituted alkyl group and *n* is 1.

6. Esters according to Claim 5, in which R represents an alkyl, aralkyl or acylalkyl group.

7. Esters according to Claim 5, in which R represents an alkyl, aralkyl or arylcarbonylakyl group.

8. Esters according to Claim 5, in which R represents an alkyl group, a substituted or unsubstituted benzyl group or a substituted or unsubstituted phenacyl group.

9. Esters according to Claim 5, in which R represents an alkyl group, a benzyl group, a benzyl group having one or more alkyl, alkoxy or halogen substituents, a phenacyl group or a phenacyl group having one or more alkyl, alkoxy or halogen substituents.

10. Esters according to Claim 5, in which R represents a methyl, ether, butyl or benzyl group.

11. A process for preparing Monacolin K salts and esters which comprises salifying or esterifying Monacolin K or a reactive derivative thereof.

12. A process according to Claim 11, in which said reactive derivative is the Monacolin K parent acid or a Monacolin K salt.

13. A process according to Claim 11, in which Monacolin K esters are prepared by reacting a halide of formula RX (wherein R represents a substituted or unsubstituted alkyl group and X represents a halogen atom) with a Monacolin K salt.

14. A process according to Claim 11, in which Monacolin K esters are prepared by reacting an alcohol of formula ROH (wherein R represents a substituted or unsubstituted alkyl group) with Monacolin K or its parent acid in the presence of a dehydrating agent.

15. A process for preparing Monacolin K salts, which comprises cultivating a Monacolin K salt-producing microorganism of the genus *Monascus* in a culture medium therefor and separating a Monacolin K salt from the culture medium.

16. A process according to Claim 15, in which said microorganism of the genus *monascus* is: *Monascus anka* SANK 10171 (IFO 6540); *Monascus purpurous* SANK 10271 (IFO 4513); *Monascus ruber* SANK 10671 (Ferm 4958); *Monascus vitreus* SANK 10960 (NIHS 609, e-609; Ferm 4960); *Monascus paxii* SANK 11172 (IFO 8201); *Monascus ruber* SANK 11272 (IFO 9203); *Monascus ruber* SANK 13778 (Ferm 4959); *Monascus ruber* SANK 15177 (Ferm 4956); *Monascus ruber* SANK 17075 (CBS 832.70); *Monascus ruber* SANK 17175 (CBS 503.70); *Monascus ruber* SANK 17275 (ATCC 18199); or *Monascus ruber* SANK 18174 (Ferm 4957).

17. A process according to Claim 15, in which said microorganism is: *Monascus ruber* SANK 10671 (Ferm 4958); *Monascus ruber* SANK 11272 (IFO 9203); *Monascus ruber* SANK 13778 (Ferm 4959); *Monascus ruber* SANK 15177 (Ferm 4956); or *Monascus ruber* SANK 18174 (Ferm 4957).

18. A process according to any one of Claim 15 to 17, in which said metal is sodium, potassium, calcium, magnesium, aluminium, iron, zinc, copper, nickel or cobalt.

19. A process according to Claim 18, in which said metal is sodium, calcium or aluminium.

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